SUPPLEMENTAL DATA

1. Methods
2. Glossary of terms
3. References
4. Figures (with legends)

a.

METHODS

Animals: Lobsters (*Homarus americanus*) were purchased at the Trenton Bridge Lobster Pound, Trenton, MA. Earthworms (*Lumbricus terrestris*) were purchased at a Mobil gas station on Route 12, Northfield, CT. Adult amphioxus (*Branchiostoma floridae*) were purchased from Gulf Specimen Marine Lab (Panacea, FL). Adult Atlantic hagfish (*Myxine glutinosa*), were purchased from a commercial supplier (Huntsman Marine Science Center, St Andrews, NB, Canada) who caught the specimens in the Bay of Fundy, Canada. Hagfish were maintained in the dark in circular tanks with running seawater at 10±2°C. The animals were anesthetized in seawater containing tricaine methanesulfonate (MS 222; Sigma-Aldrich, St Louis, MO).

Histochemistry and electron microscopy: Plastic sections (1 μm) were stained with alkaline Giemsa stain for study by light microscopy, and 70- to 80-nm thin sections were examined by electron microscopy with Philips 300, 400, and CM10 electron microscopes (Philips, Eindhoven, The Netherlands) by 3 different microscopists, as previously described [1].

GLOSSARY

Amoebocyte. A type of hemocyte that adheres to the basement membrane lining of invertebrate blood vessels. A possible evolutionary precursor of the endothelial cell.

Chordates. A phylum with a dorsal, hollow neural tube and a notochord.

Blood vascular systems (aka hemal system). Systems of internal fluid transport of coelomate metazoans formed within extracellular matrix, limited by basement membranes of endodermal, mesodermal and ectodermal epithelia. They are generally unlined by cells and major components are usually dorsal-ventral in anatomical position. Comprised of vessels, sinuses (saclike cavities) or hemocoel (large sinus that becomes the principle body cavity) [2].

Circulatory system. Any system of moving fluids that reduces the functional diffusion distance that nutrients, gases and metabolic waste products must traverse regardless of its embryological origin or its design. Provides a mass flow of fluid (i.e., convection, bulk flow), freeing the animal from the body-size and –shape limitations imposed by simple diffusion.

Closed circulatory system. An anatomically defined compartment separated from the coelomic cavity, consisting of a network of continuous vessels in which blood never comes in contact with the cells of the surrounding tissue. In invertebrates, comprises

simple channels surrounded by basal cell surfaces and extracellular matrix of surrounding epithelial cells; in vertebrates, consists of multicellular tubes with central lumen, surrounded by the apical surface of endothelial cells.

Coelom (aka secondary body cavity, the primary being the blastocoel). A body cavity that in invertebrates may occupy all the spaces between organs.

Coelomates. Animals with a coelom.

Coelomic circulatory systems. Systems of internal fluid transport of coelomate metazoans formed by mesodermally derived body cavities. The cavities are generally right and left in anatomical position and are lined by cells with their apical surfaces directed towards the lumen [2].

Coelomocytes. Free circulating cells in the coelomic space of invertebrates.

Convergent evolution. Independent evolution of similar appearing structures in entirely unrelated groups of organisms. Also referred to as analogous function or homoplasy, which is defined as the presence of a similar character in two different animals by a different derivation, not as the result of inheritance of that character from a common ancestor [3].

Diploblastic animals. Animals with two germ layers (ectoderm and endoderm).

Ecdysozoans. A group of protostome animals that periodically shed their cuticles.

Endothelium. A more or less continuous layer of epithelial cells interconnected by specialized junctional complexes lining the inner surface of blood vessels. The cells display basoapical polarity (with apical surface facing the lumen) and are anchored to the basement membrane.

Hemocytes. Cells that are present within invertebrate blood vascular systems.

Hemocoel. The body cavity and spaces/sinuses between the organs of most mollusks and arthropods though which blood circulates.

Hemolymph. A term used to describe the blood in open circulatory systems.

Homologous structures. Characters that are present in two or more taxa, but are traceable phylogenetically and ontogenetically to the same character in the common ancestor of those taxa [4]. Stated another way, homology is defined as the occurrence of any given character in two organisms whose common ancestor also possessed the character [3]. Characters that share descent from a common ancestor are called homologues [4]. Genes and structures, but not function can be homologous [4].

Invertebrates. Metazoa that do not possess a backbone or vertebral column.

Lophotrochozoans. A group of protostome animals that share a ciliated trocophore larvae.

Mesothelium. Mesodermally derived lining of the body cavity (coelom). Their basal surface (and basal lamina) faces the lumen of blood vessels in invertebrates. Around blood vessels, they may differentiate into contractile myoepithelial cells that provide propulsion.

Metazoa (aka kingdom Animalia). Multicellular, ingestive, heterotrophic (i.e., consume food) eukaryotes. Over a million species have been described. Arose as a monophyletic group from a protist ancestor approximately 600-700 mya. Distinct from protozoans, which are unicellular eukaryotes that often form multicellular colonies.

Monophyletic group. A group of species that includes an ancestral species and all of its descendants (i.e., a single evolutionary lineage).[4]

Myoepithelial cells. A muscular differentiation of the mesothelial cells that constitute the vascular wall of some vessels in invertebrates.

Open circulatory system. Only a single compartment (hemocoel) filled with body fluid, called the hemolymph. The hemocoel is an enlargement of the connective tissue compartment (a large blood vessel/sinus) and is associated with regression of coelom.

The hemocoel lacks a lining of cells or basement membrane. Coelomata dissociate and permanent mesothelial layers lining the body cavity do not form. In an open system, blood bathes tissues directly as it percolates though a network of sinuses and lacunae.

Paraphyletic group. A group whose member species are all descendants of a common ancestry, but that does not contain all the species descended from that ancestor [4].

Surface to volume dilemma. A change in body size disproportionately changes the SA:volume ratio. As a solid 3D body becomes larger, its SA increases in proportion to the radius squared, but its volume increases more rapidly (radius cubed). Eventually, body will reach a size where SA is inadequate to serve its volume.

Triploblastic animals. Animals with three germ layers (ectoderm, endoderm and mesoderm).

Vertebrates (subphylum Vertebrata, phylum Chordata). Metazoa that possess a backbone or vertebral column. Distinguished from other members of the Chordata phylum by presence of a neural crest and a cartilaginous or ossified endoskeleton. Includes gnathostomes (jawed vertebrates), lamprey and hagfish. Comprise less than 5% of all described animals.

REFERENCES

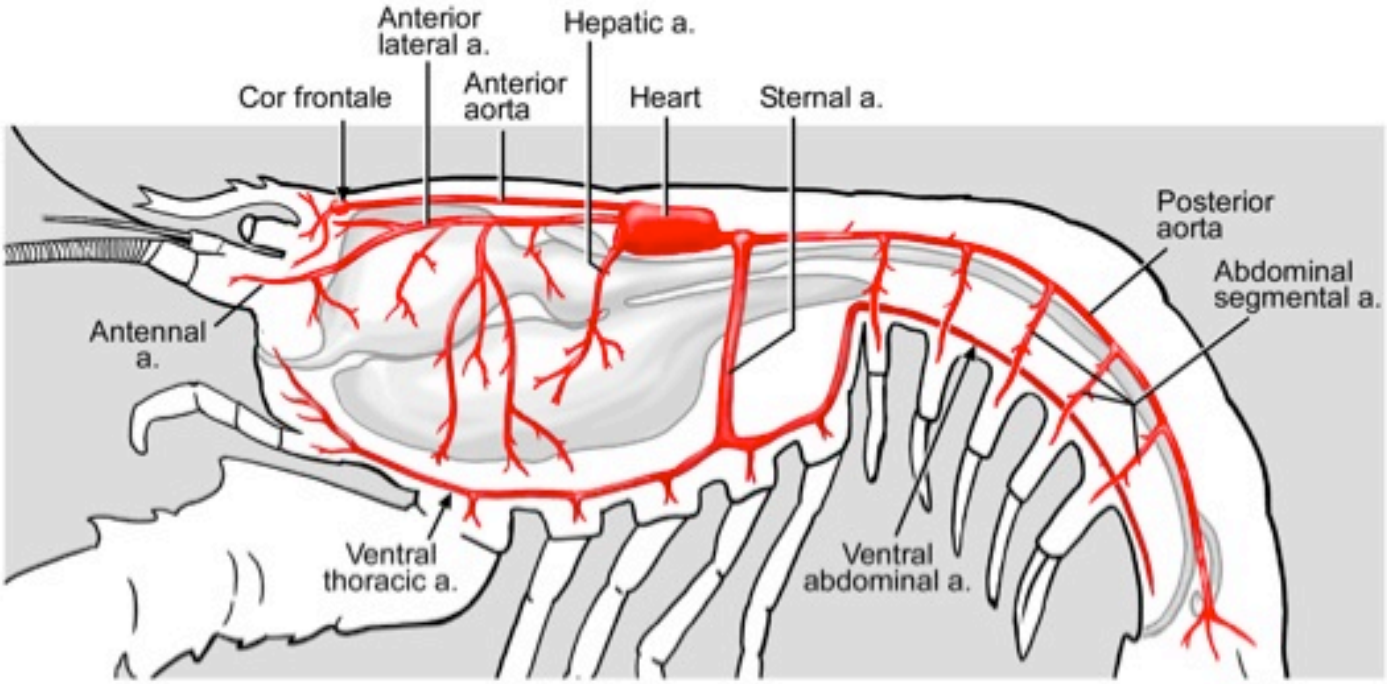
1 Dvorak AM. Monograph-Procedural guide to specimen handling for the ultrastructural pathology service laboratory. *J Electron Microsc Tech*. 1987; **6**: 255-301.

2 Ruppert EE, Carle KJ. Morphology of metazoan circulatory systems. *Zoomorphology*. 1983; **103**: 193-208.

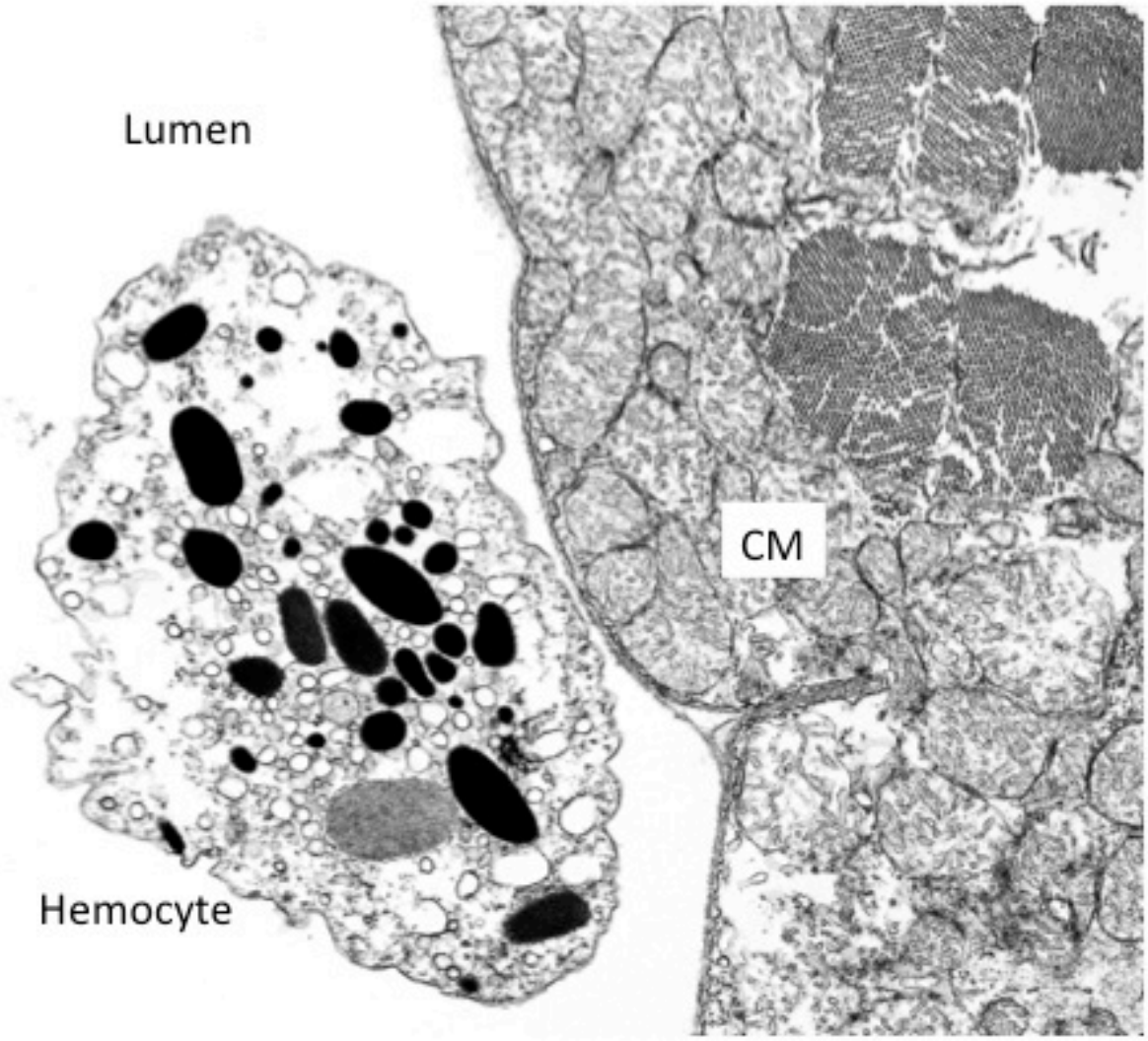
3 Xavier-Neto J, Castro RA, Sampaio AC, Azambuja AP, Castillo HA, Cravo RM, Simoes-Costa MS. Parallel avenues in the evolution of hearts and pumping organs. *Cell Mol Life Sci*. 2007; **64**: 719-34. 10.1007/s00018-007-6524-1.

4 Brusca RC, Brusca GJ. *Invertebrates*. Sunderland, Mass.: Sinauer Associates, 2003.

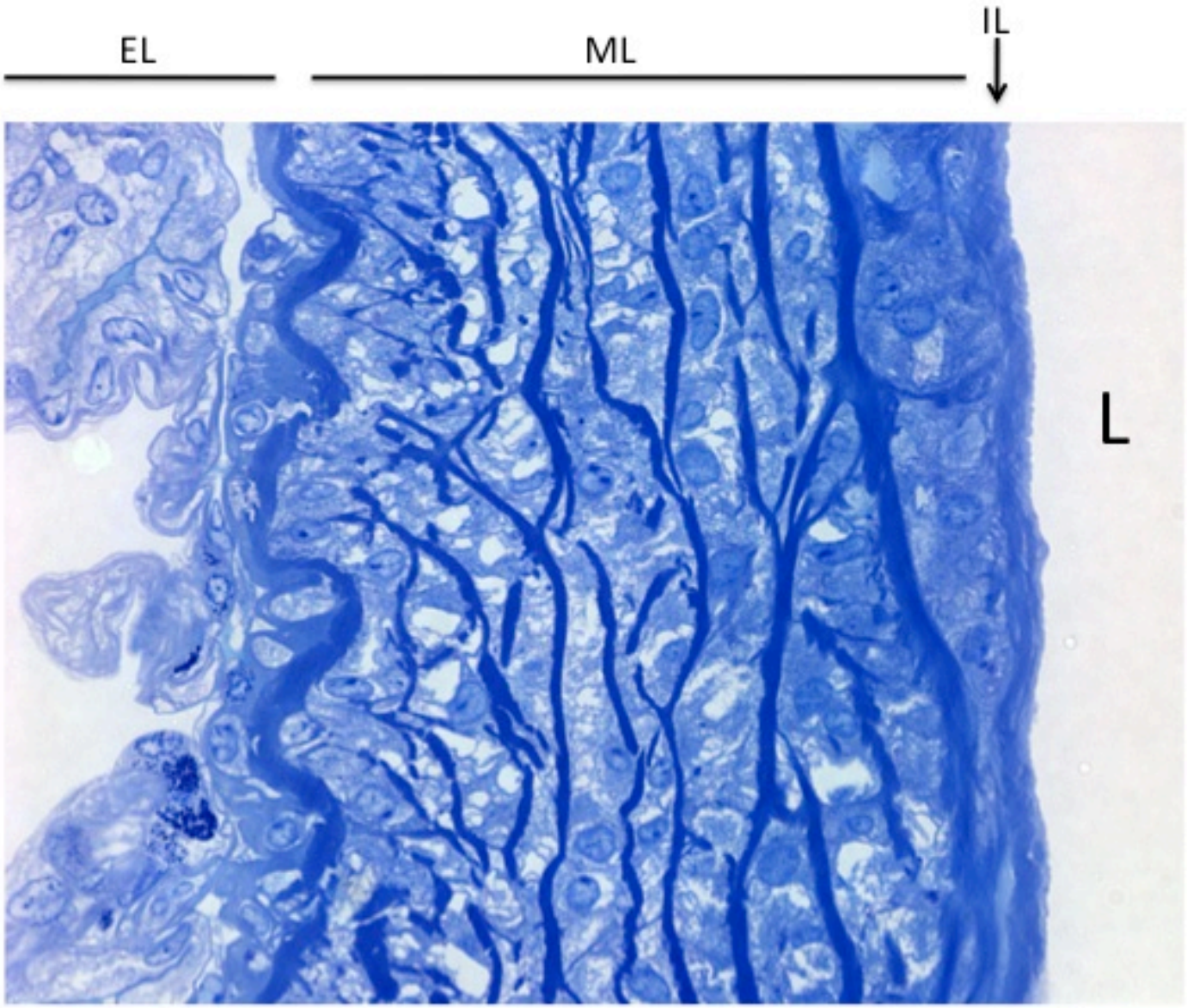
5 Ruppert EE, Fox RS, Barnes RD. *Invertebrate zoology : a functional evolutionary approach*. Belmont, CA: Thomson-Brooks/Cole, 2004.



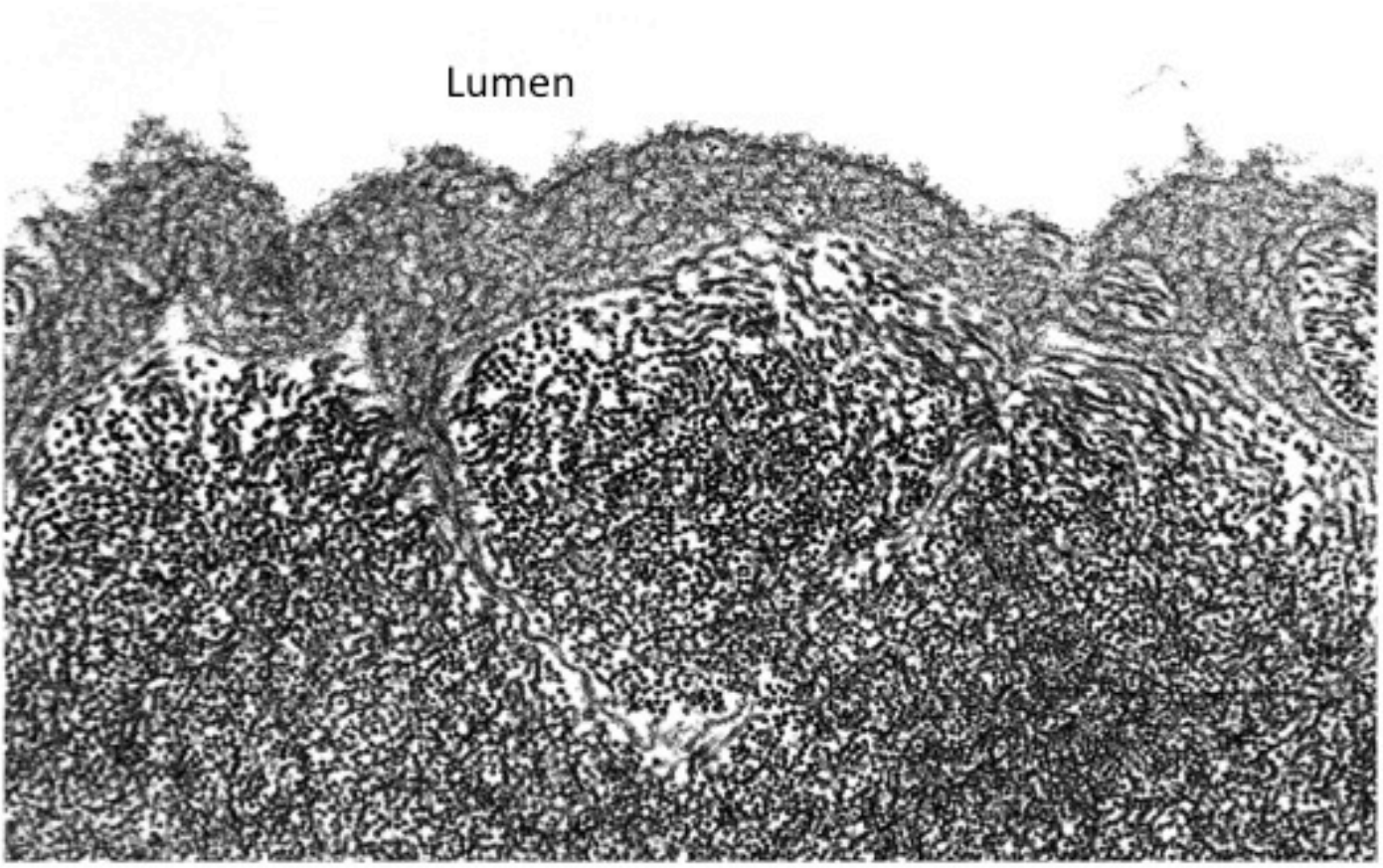
Suppl. Fig. I. Schematic of lobster circulation. a, artery. Enlargement of Fig. 3A.



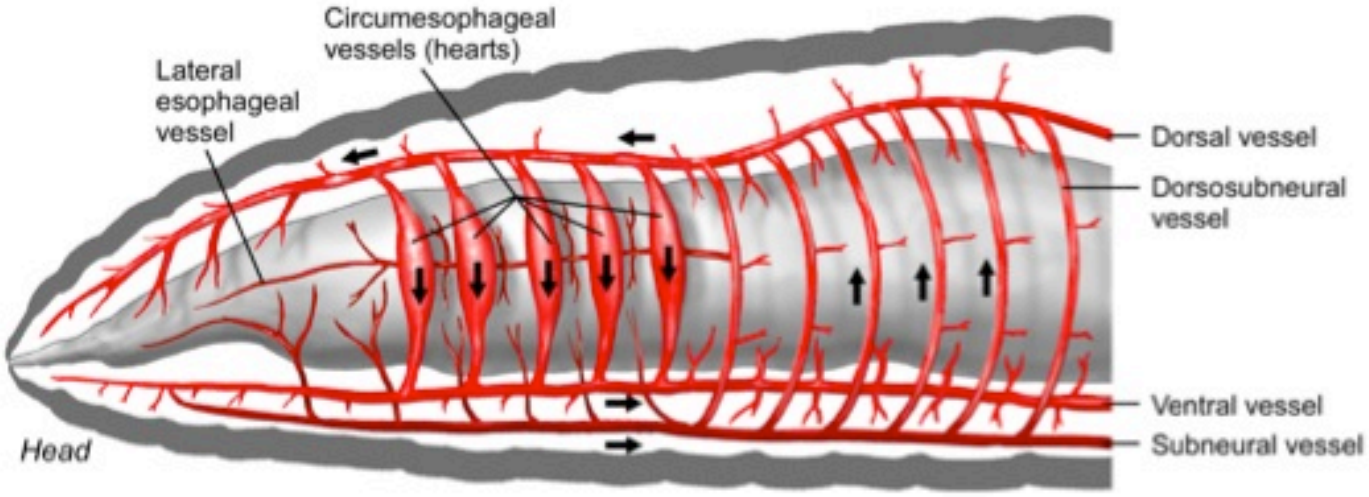
Suppl. Fig. II. Electron micrograph of the lobster heart. CM, cardiomyocyte. Enlargement of Fig. 3C.



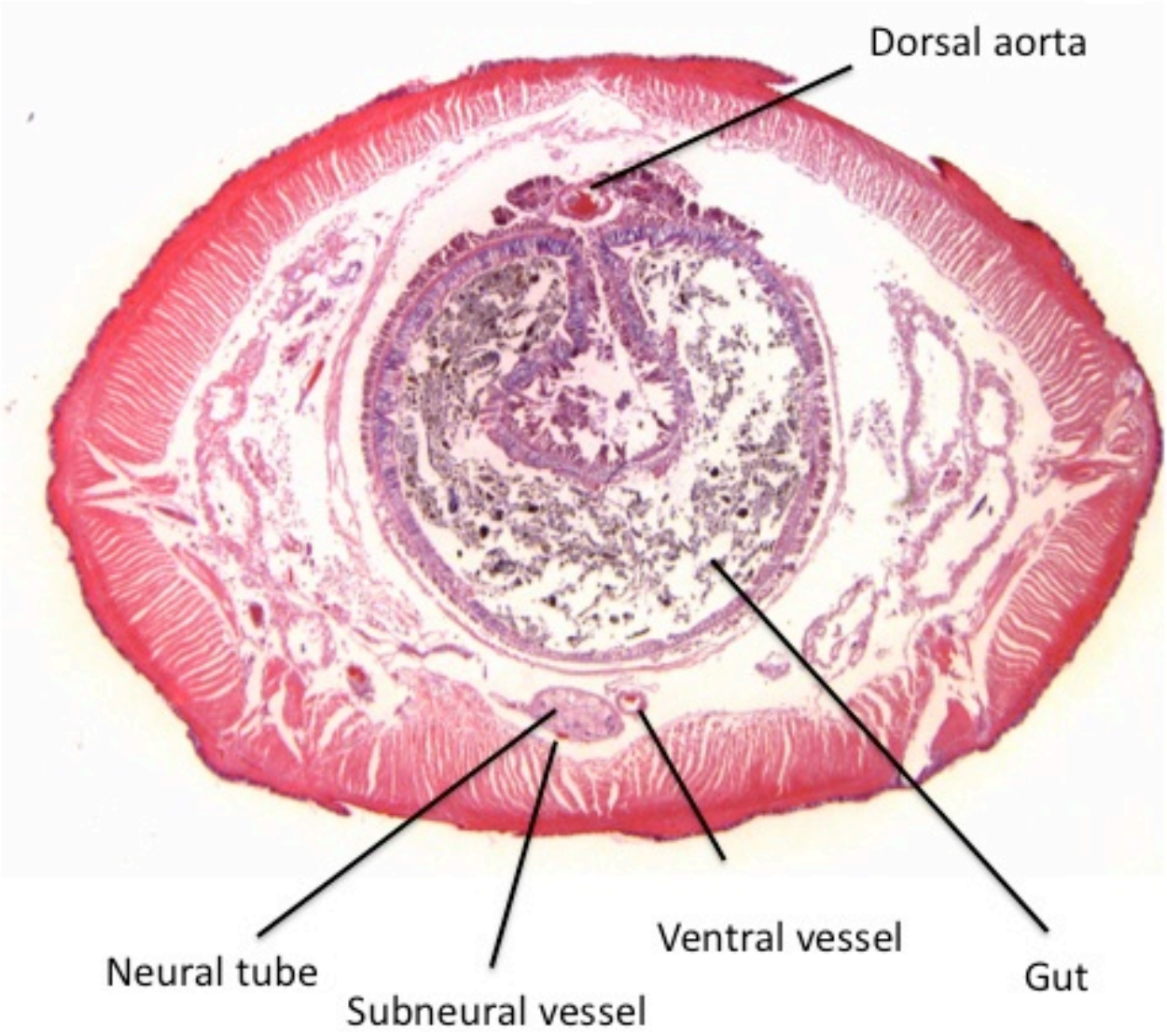
Suppl. Fig. III. A one-micron Giemsa-stained cross-section of the dorsal abdominal wall of the lobster showing three layers: an acellular internal lamina (IL) next to the lumen (L), a middle lamina (ML) containing cells (fibroblasts?) and an external Lamina (EL). Enlargement of Fig. 3D.



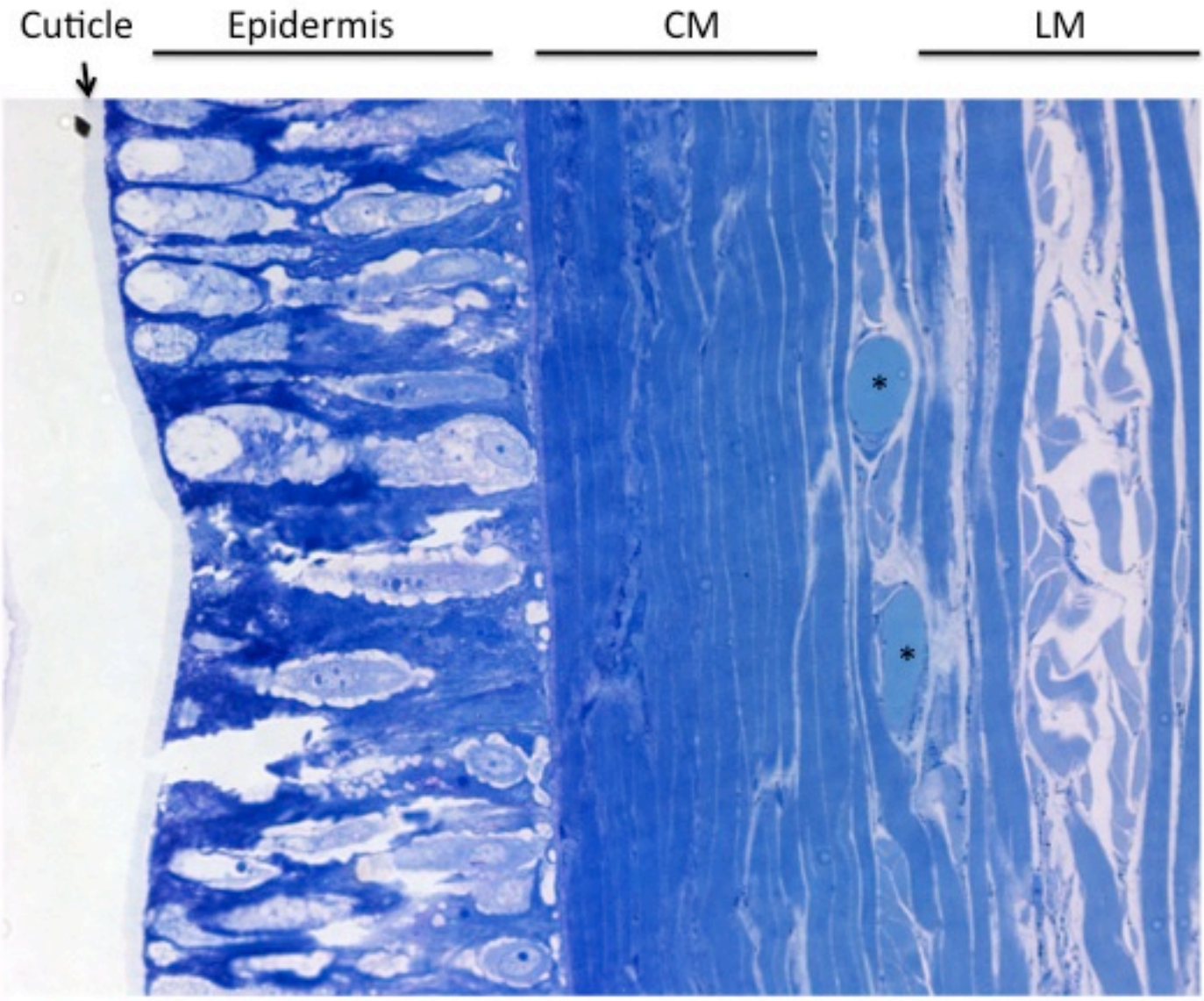
Suppl. Fig. IV. Electron micrograph of the lobster aorta. Enlargement of Fig. 3E.



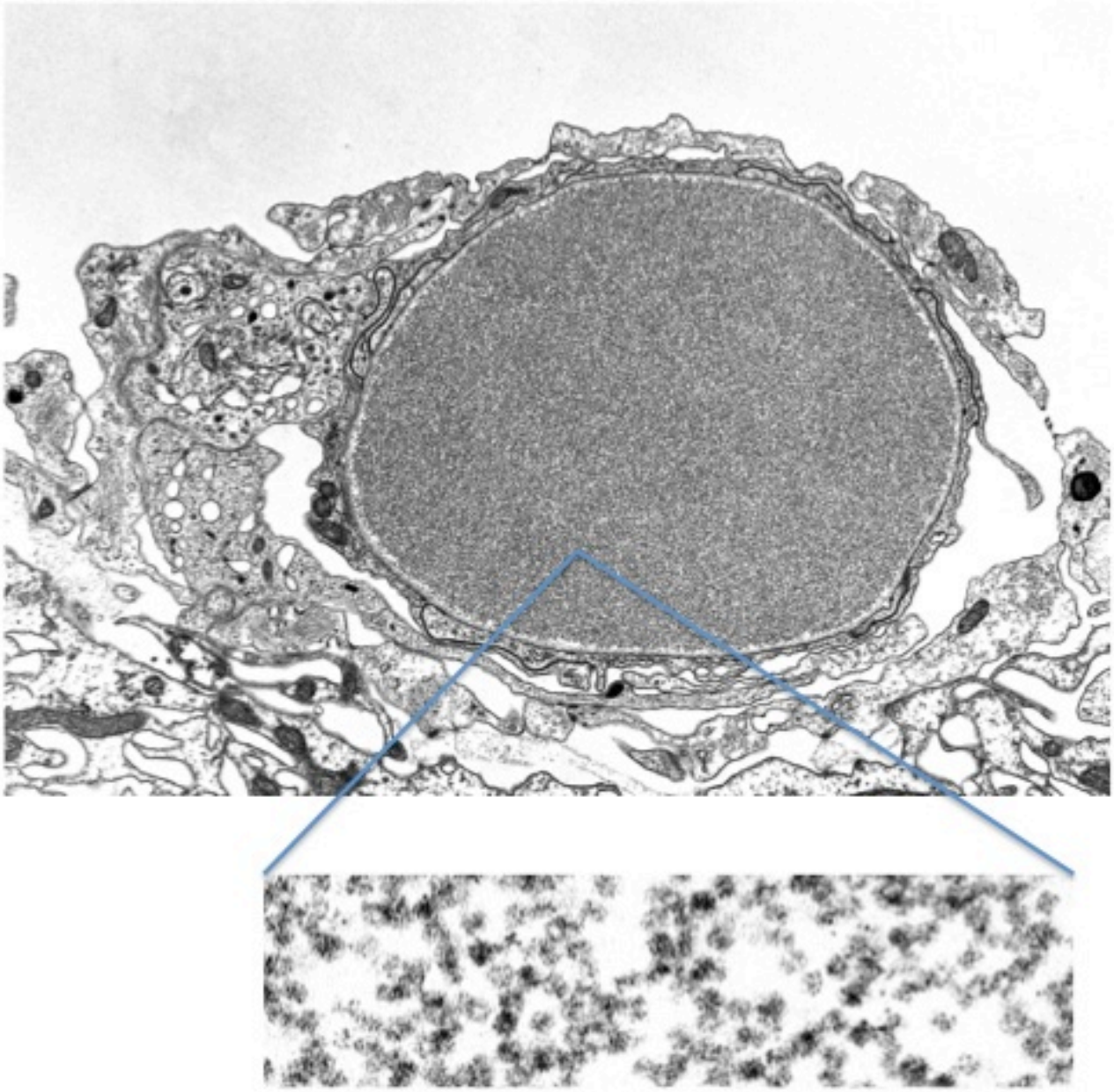
Suppl. Fig. V. Schematic of earthworm circulation. Enlargement of Fig. 4A.



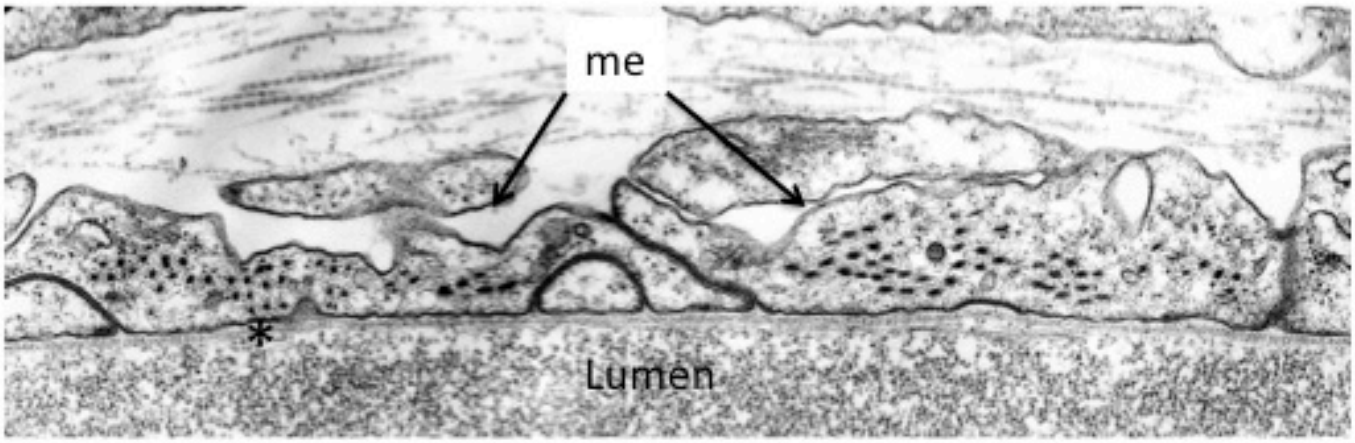
Suppl Fig. VI. Transverse histological section through the body of the earthworm shows the dorsal vessel (arrow) on the dorsal side of the gut. The section was stained with H&E. Enlargement of Fig. 4B.



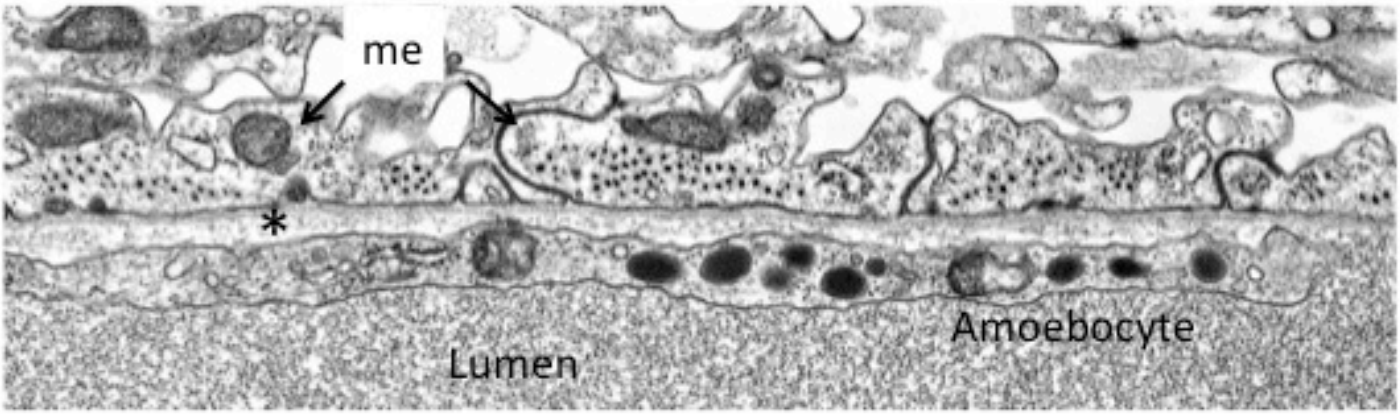
Suppl. Fig. VII. A one-micron section Giemsa-stained cross-section of the body wall of the earthworm showing cuticle, epidermis, circular muscle layer (CM), and longitudinal muscle layer (LM) containing two small blood vessels (*). Enlargement of Fig. 4C.



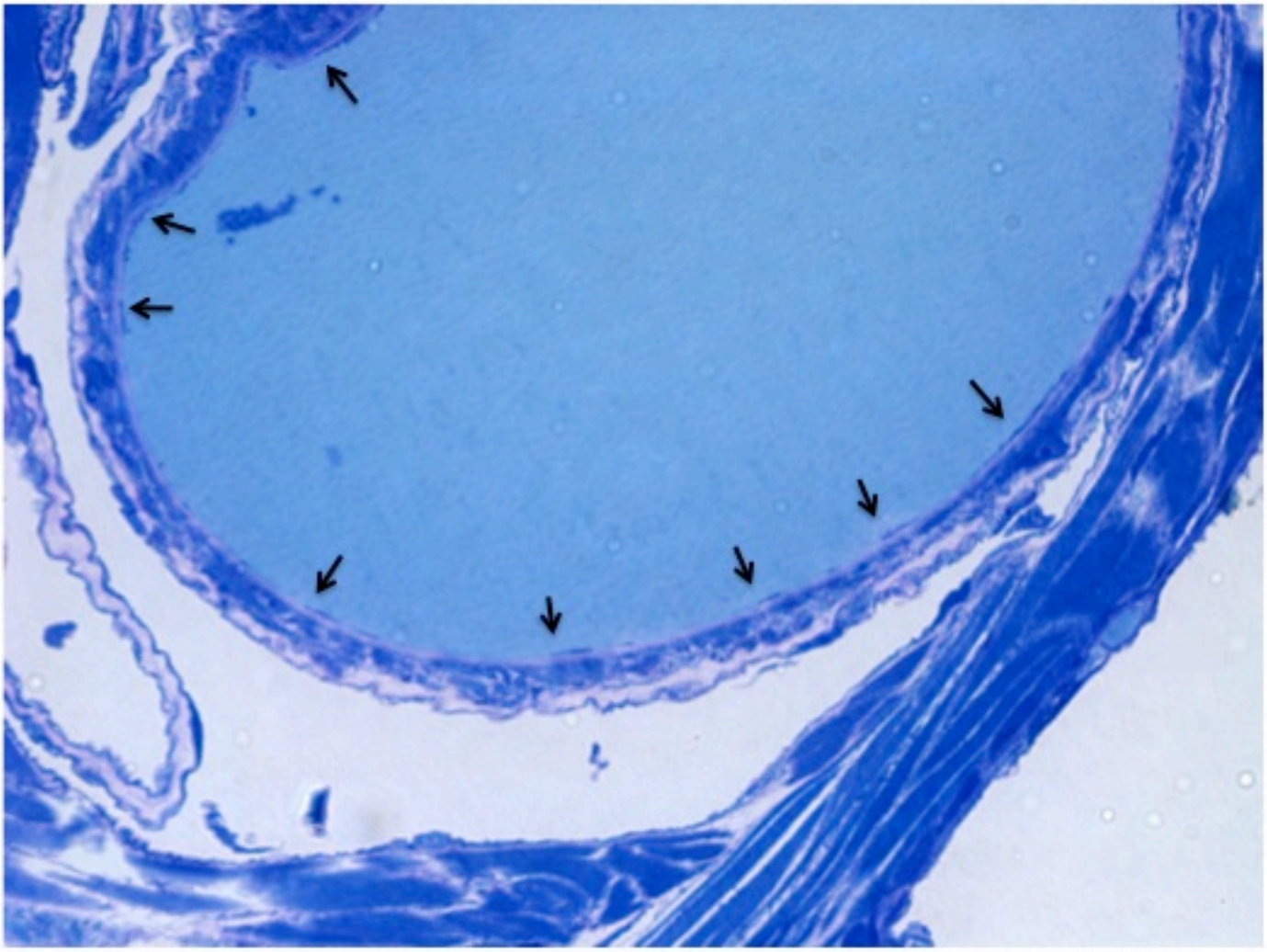
Suppl Fig. VIII. Electron micrograph of a small blood vessel surrounded by a continuous layer of myoepithelial cells. The lumen is filled with hemoglobin particles (shown at higher magnification in lower panel). Enlargement of Fig. 4C.



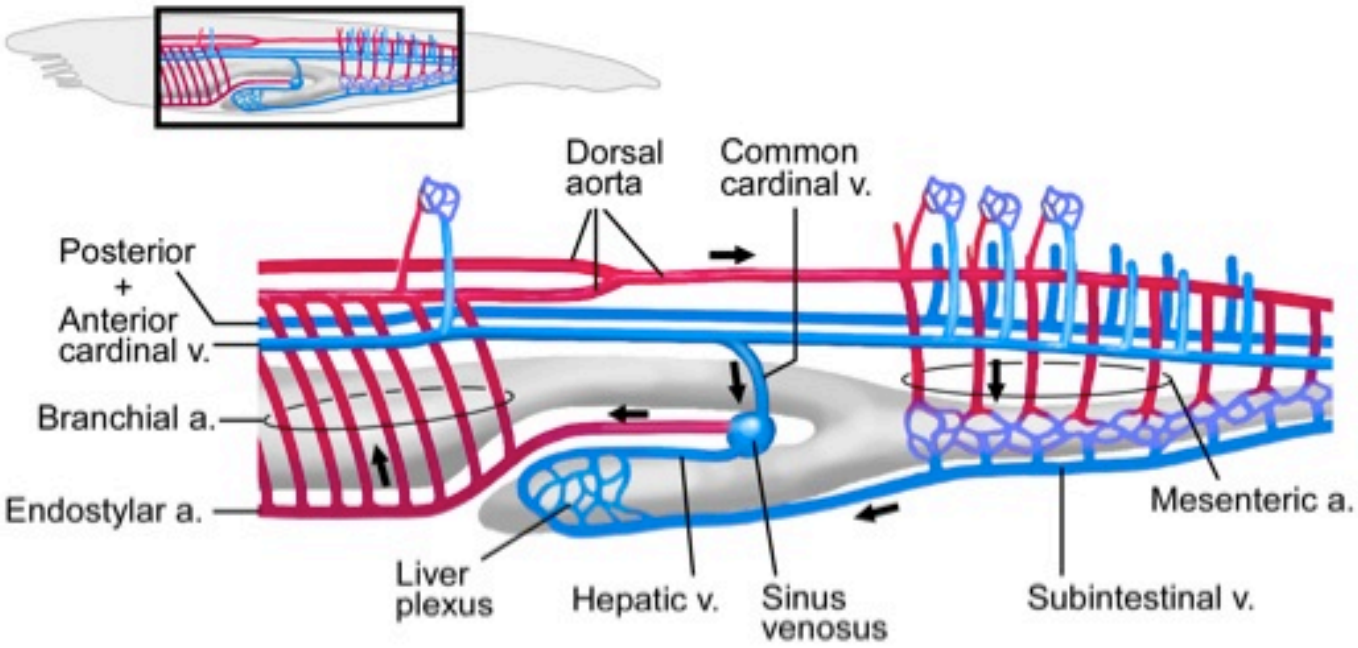
Suppl. Fig. IX. Electron micrograph shows a blood vessel lined by several myoepithelial cells (me). The cells are connected by specialized lateral borders. They contain numerous thick myofilaments (arrows) consistent with myosin that are oriented circumferentially around the vessel. A well-formed basal lamina (*) separates the myoepithelial cells from the lumen of the blood vessel. Enlargement of Fig. 4E.



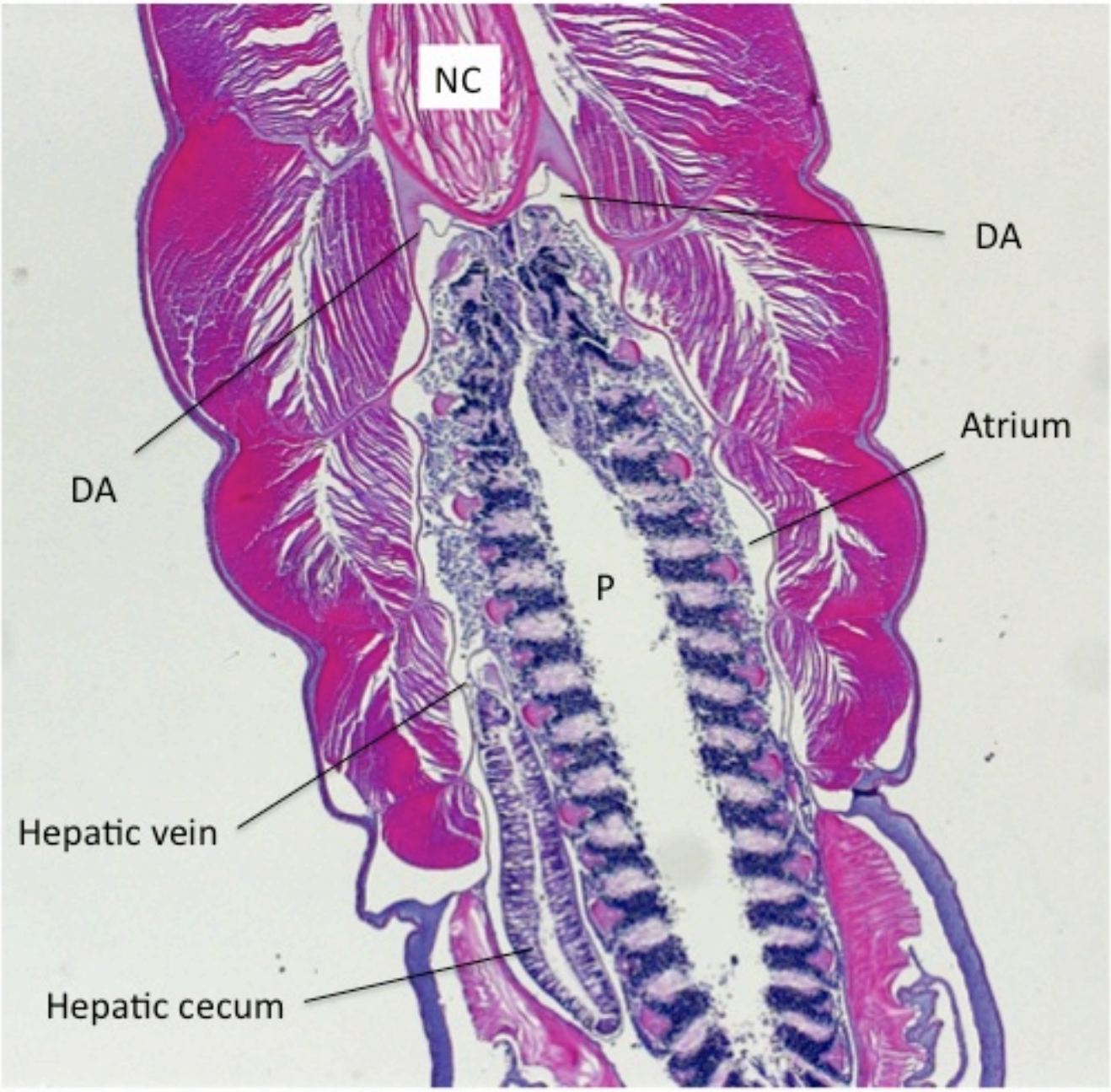
Suppl. Fig. X. Electron micrograph of a blood vessel from the earthworm showing a dense-granule-laden amoebocyte adhering to the inner side of the lumen. me, myoepithelial cells. Enlargement of Fig. 4F.



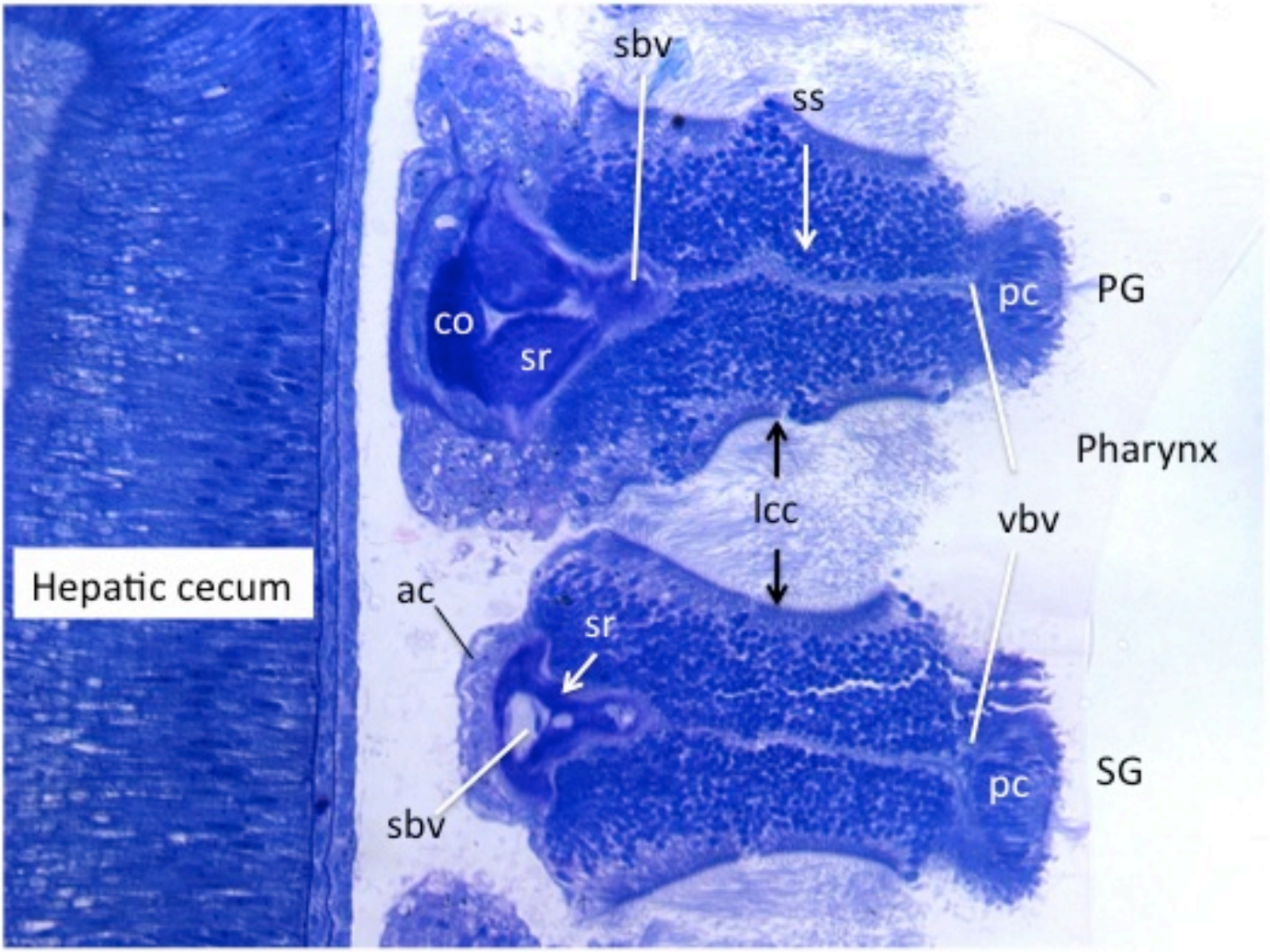
Suppl. Fig. XI. A one-micron Giemsa-stained cross-section of a blood vessel in the earthworm reveals a number of amoebocytes adhering to the inner surface of the lumen (arrows).



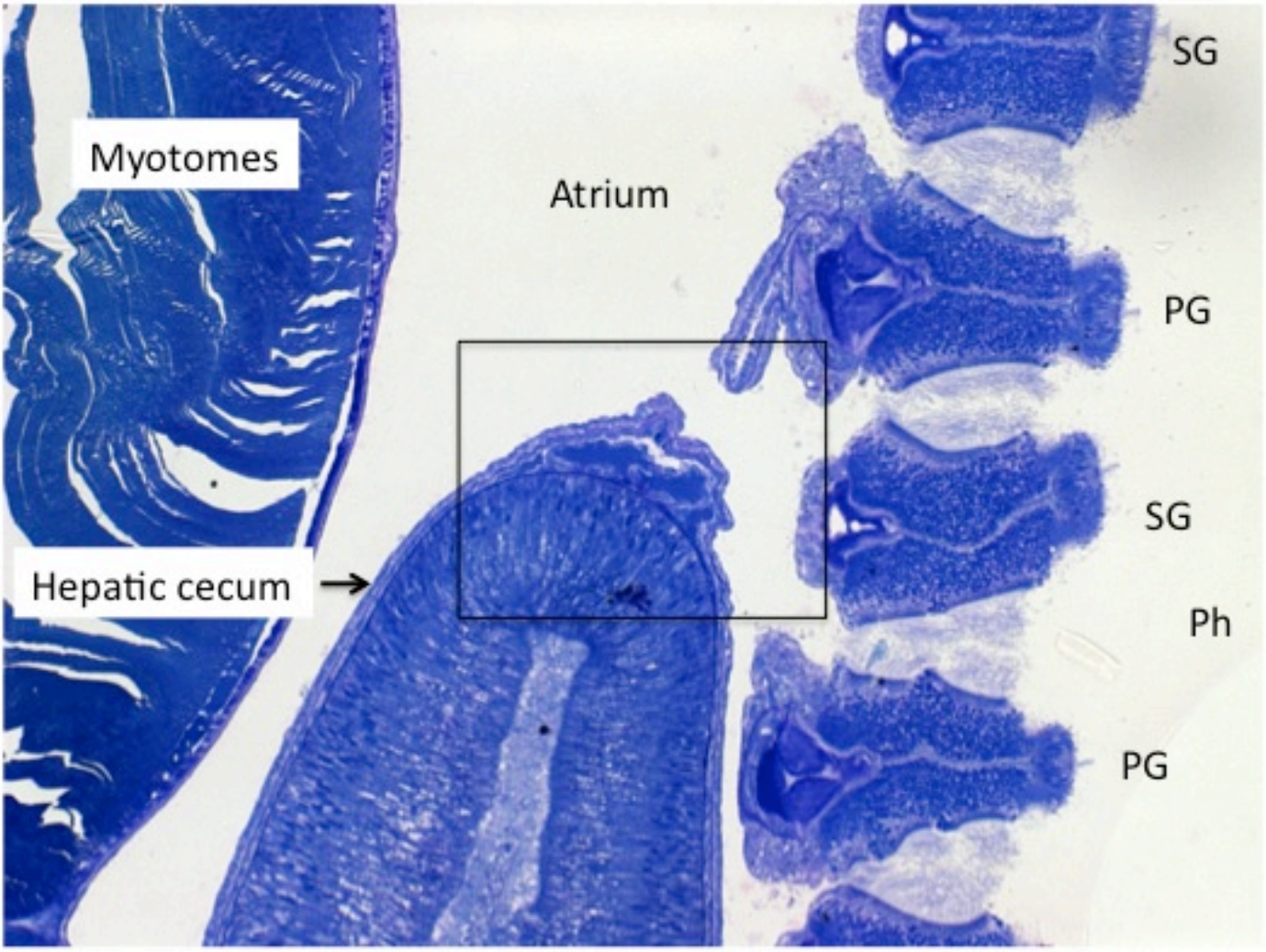
Suppl. Fig. XII. Schematic of amphioxus circulation. A, artery; v, vein. Enlargement of Fig. 5A.



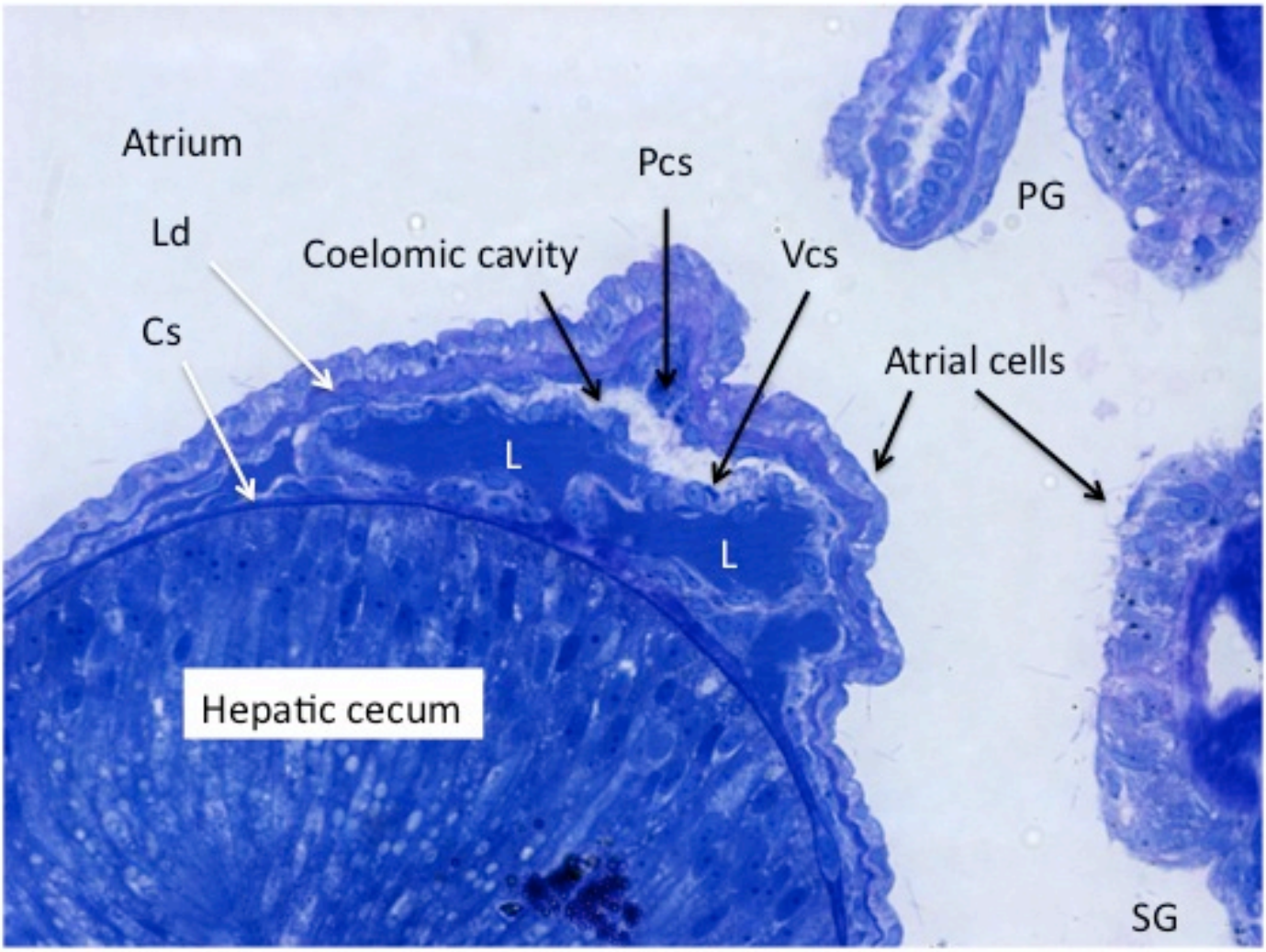
Suppl. Fig. XIII. A five-micron H&E-stained cross-section through the pharynx of amphioxus. NC, notochord, DA; dorsal aorta; P, pharynx. Enlargement of Fig. 5B.



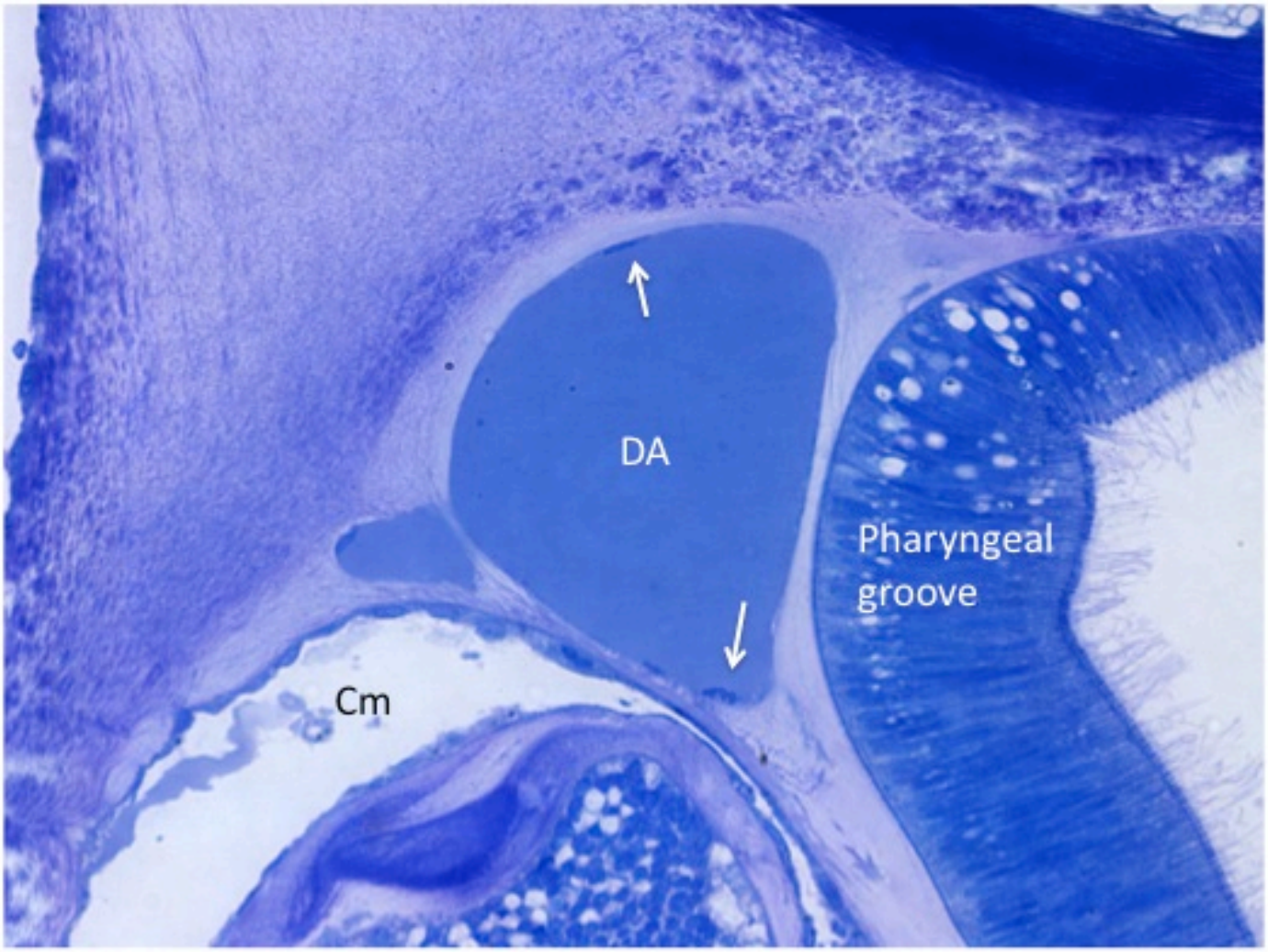
Suppl. Fig. XIV. A one-micron Giemsa-stained cross-section of gills in amphioxus. Primary gill bar (PG) is separated from secondary gill bar (SG) by a gill slit. Only the primary gill bar has a coelomic canal. The primary gill bar has three blood vessels: the skeletal blood vessel (sbv), the visceral vessel (vbv) and the coelomic vessel (not readily seen). The secondary gill bar has two vessels: the skv (which is more prominent compared with the primary gill bar) and the vbv. Ac, atrial cells; lcc, lateral ciliated cells; pc, pharyngeal cells; sr, skelatal rod; ss, stromal septum.



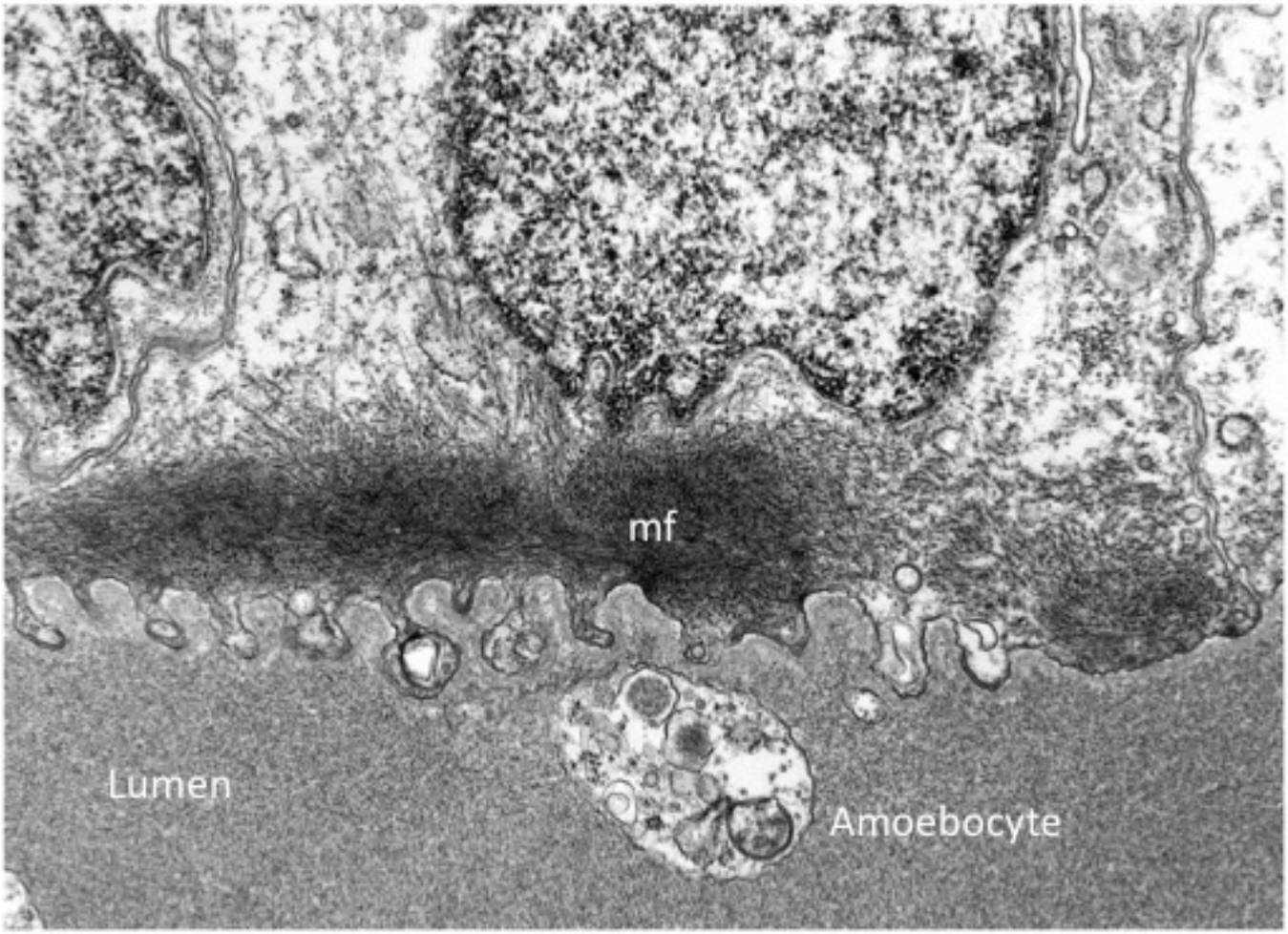
Suppl. Fig. XV. A one-micron Giemsa-stained cross-section through the mid-body region of amphioxus showing the hepatic cecum, a series of primary gill bars (PG) and secondary gill bars (SG). The hepatic vein Is located in the boxed region, which is shown at higher magnification In the next figure. Ph, pharynx.



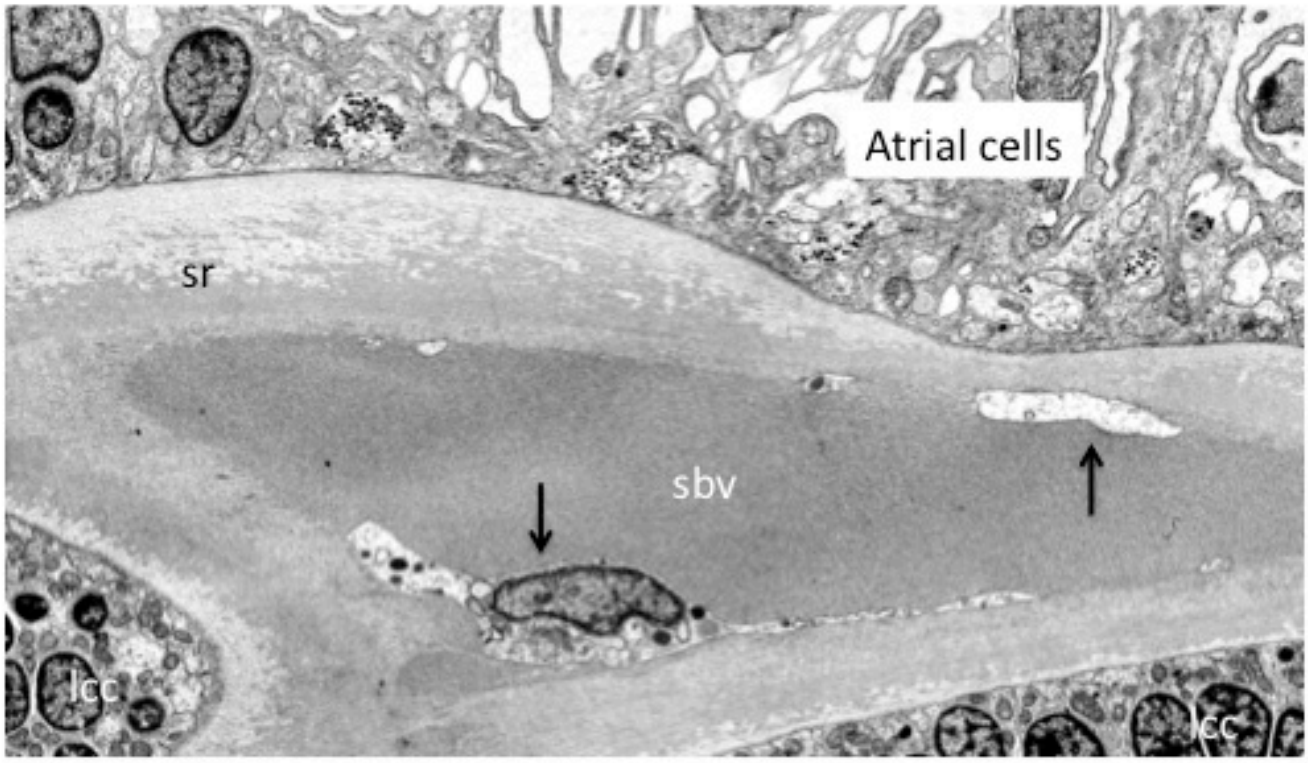
Suppl. Fig. XVI. A one-micron Giemsa-stained cross-section of the hepatic vein in amphioxus. The lumen of the hepatic vein (L) is shown. There are multiple layers between the atrium and the blood vessel, including a layer of atrial cells, a connective tissue ligament (ligamentum denticulatum; ld), parietal coelomic cells (Pcs), and visceral coelomic cells (Vsc) (which have a myoepithelial phenotype). The connective tissue around the surface of the cecum contains a circulatory sinus (cs) that is connected to the hepatic vein. PG, primary gill bar; SG, secondary gill bar.



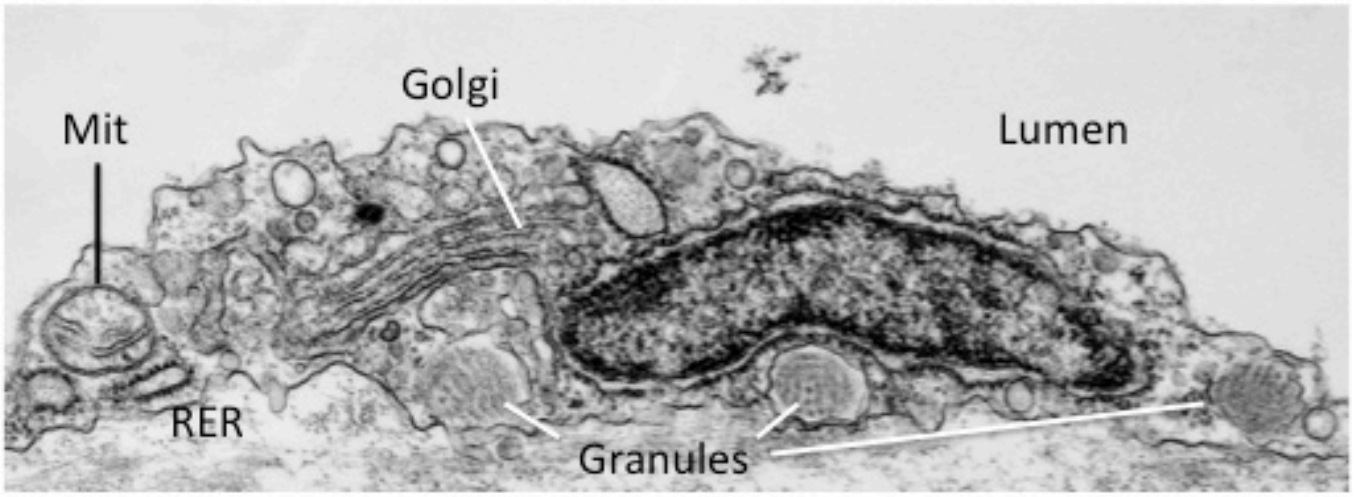
Supp. Fig. XVII. A one-micron Giemsa-stained cross-section of the dorsal aorta (DA) of amphioxus. Note occasional amoebocytes (arrows) adhering to the inner surface of the DA. CM, coelom. Enlargement of Fig. 5C.



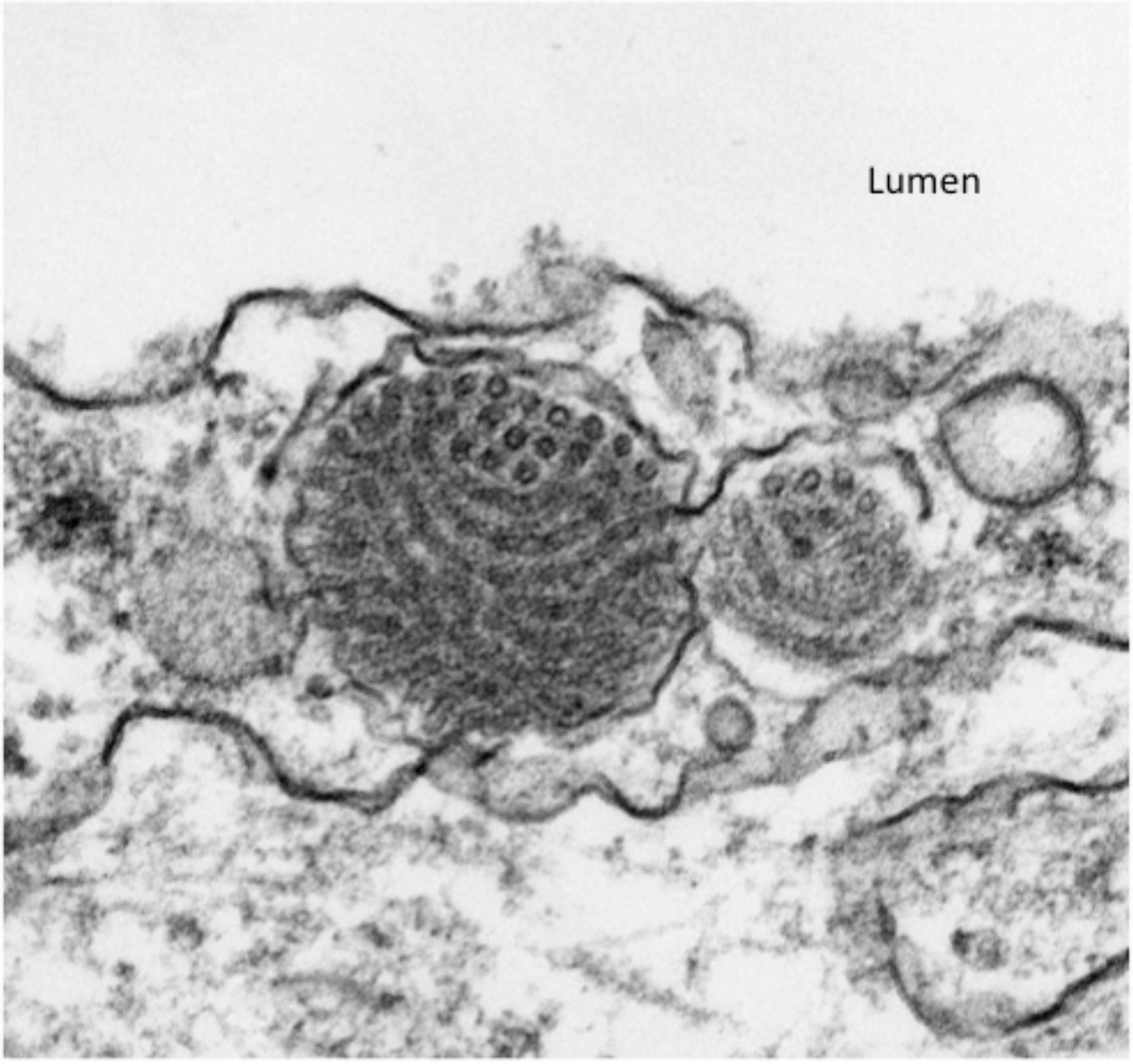
Suppl. Fig. XVIII. Electron micrograph of the contractile hepatic vein from amphioxus. Abundant myofilaments (mf) are seen on the basal side of myoepithelial cells facing the lumen. An amoebocyte is shown in the lumen. Enlargement of Fig. 5D.



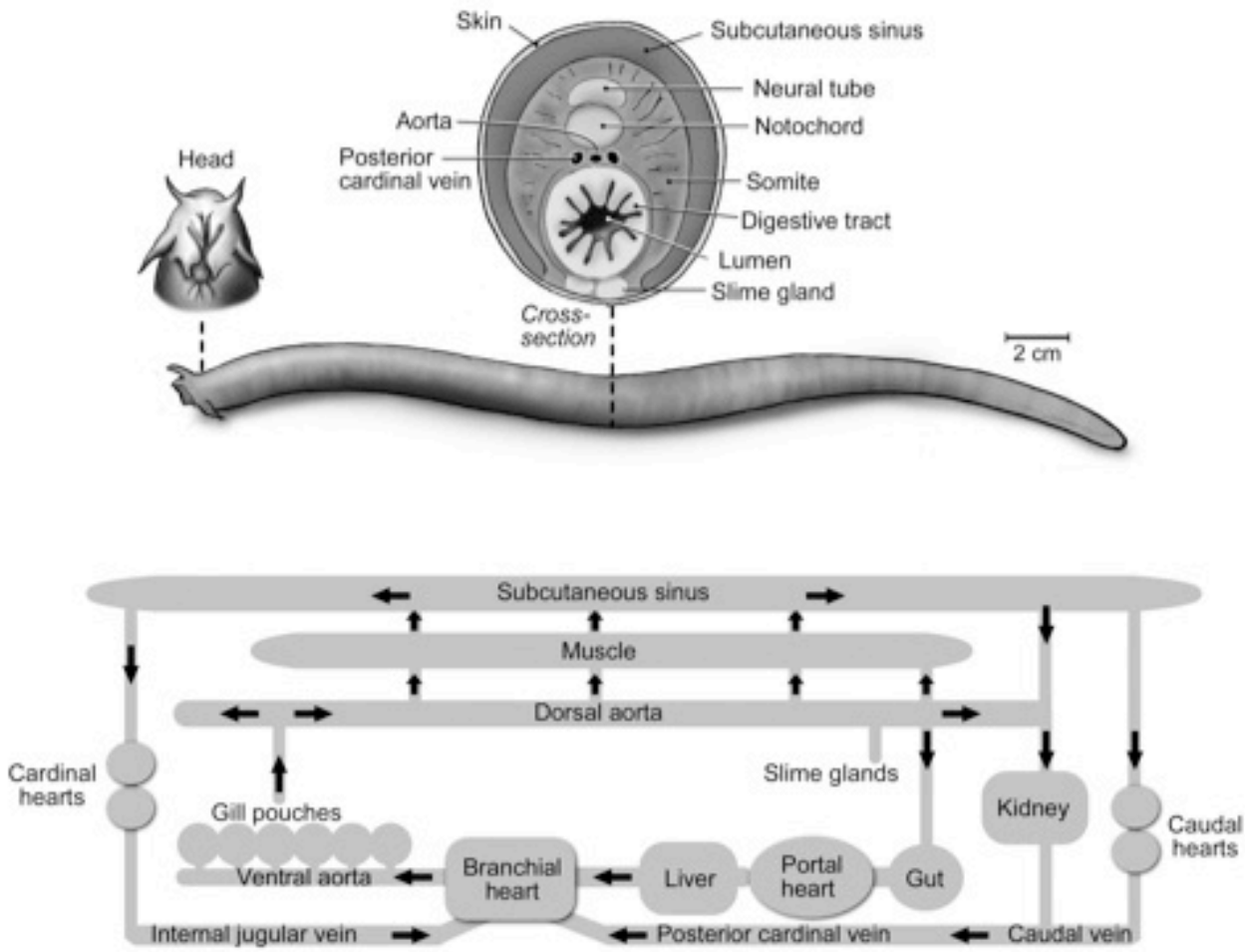
Suppl. Fig. XIX. Electron micrograph of a skeletal blood vessel (sbv) in a secondary gill bar of amphioxus. Sr, skeletal rod; lcc, lateral ciliated cells; arrows, amoebocytes. Enlargement of Fig. 5E.



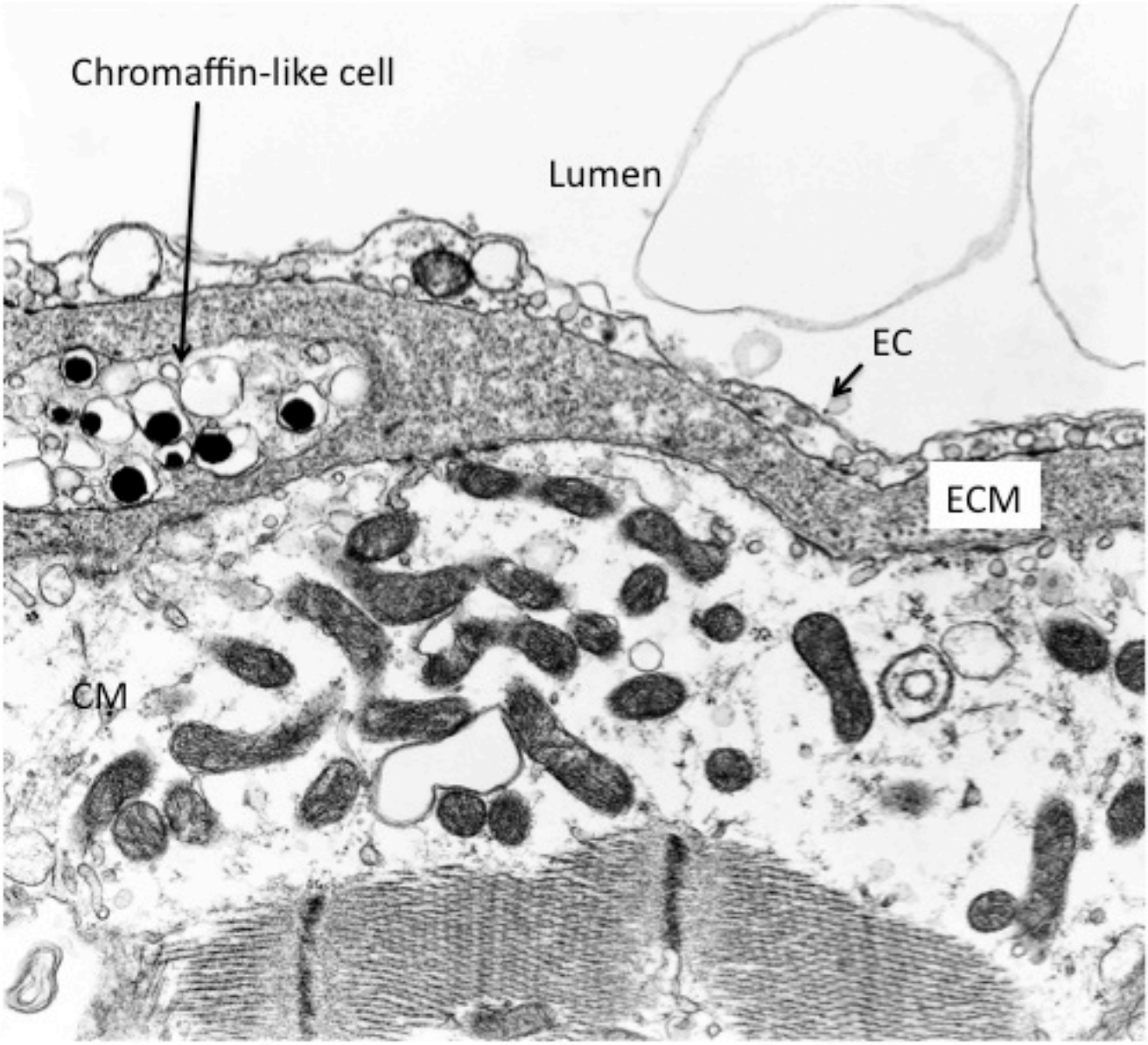
Suppl. Fig. XX. Electron micrograph of an amoebocyte adhering to the inner wall of the dorsal aorta. Shown is a prominent Golgi apparatus, rough endoplasmic reticulum (RER), mitochondrion (mit), vesicles and several tubule-filled granules.
Enlargement of Fig. 5F.



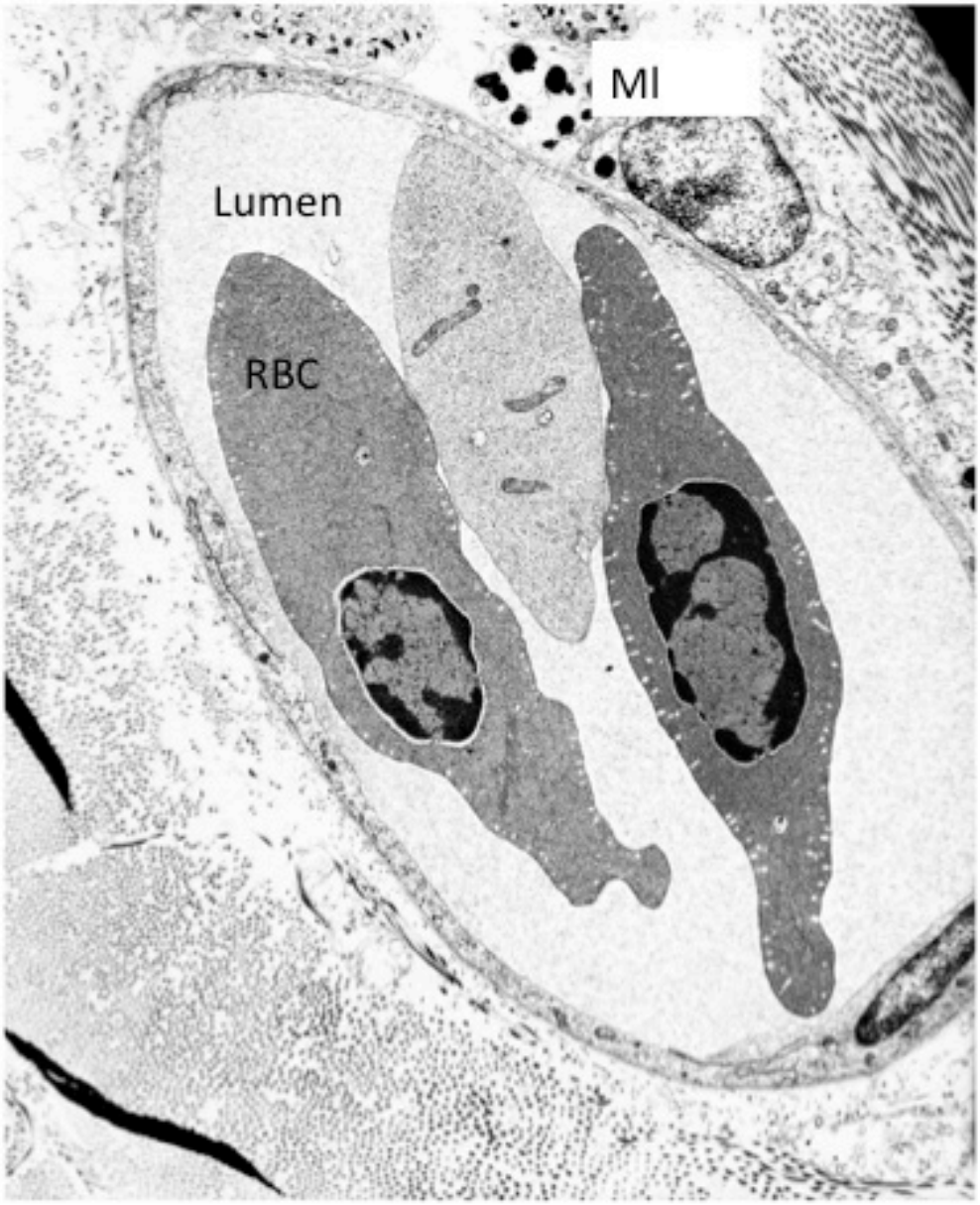
Suppl. Fig. XXI. Electron micrograph of two tubule-filled granules in an amoebocyte. Enlargement of Fig. 5G.



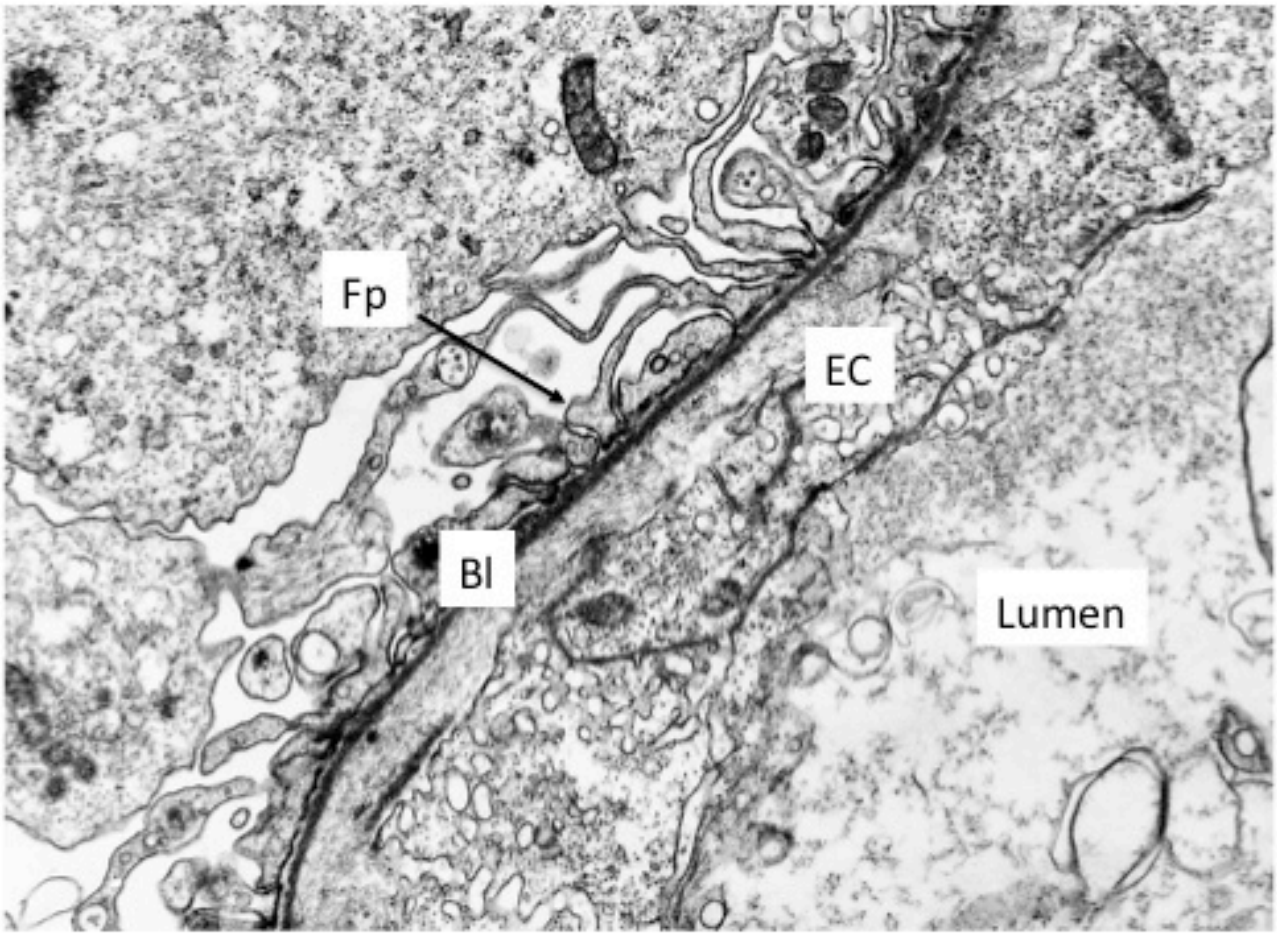
Suppl. Fig. XXII. Schematic of hagfish circulation. Enlargement of Fig. 6A.



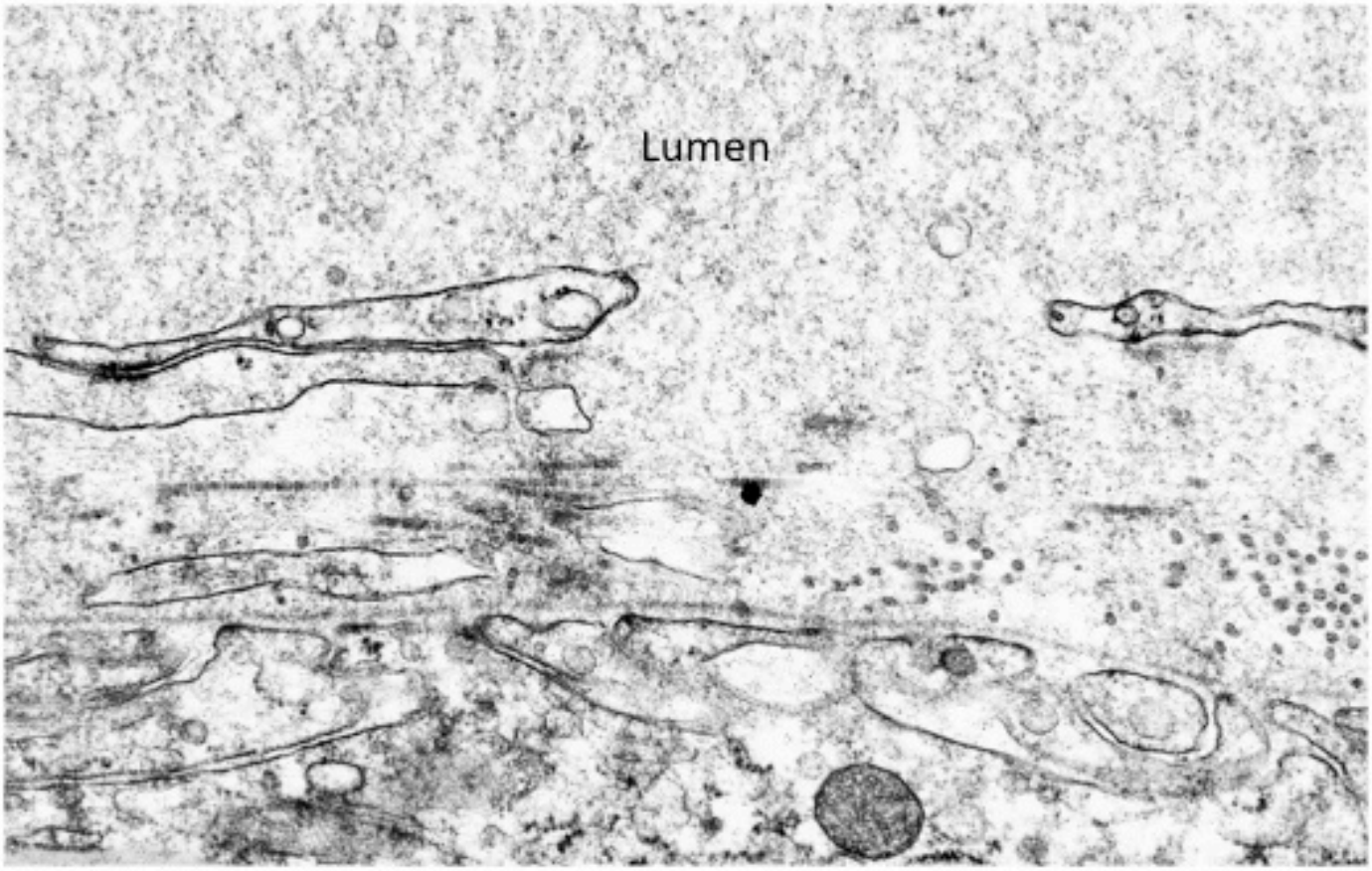
Suppl. Fig. XXIII. Electron micrograph of a heart sinus in hagfish showing an electron-lucent endothelial cell (EC) layer overlying a thick basement membrane/ extracellular matrix (ECM), followed by a cardiomyocyte (CM). Also shown is a chromaffin-like cell containing dense core secretory granules consistent with serotonin or norepinephrine content. Enlargement of Fig. 6C.



Suppl. Fig. XXIV. Electron micrograph of a dermal capillary cell; Ml, melanocyte. Enlargement of Fig. 6D.



Suppl. Fig. XXV. Electron micrograph of a a glomerular capillary from hagfish. Fp, podocyte foot processes; Bl, basal lamina; EC, endothelial cell. Enlargement of Fig. 6E.



Suppl. Fig. XXVI. Electron micrograph of an hepatic sinusoid from hagfish. Enlargement of Fig. 6F.